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DETECTION AND DETERMINATION OF SMALL QUANTITIES OF ETHYL AND METHYL ALCOHOL AND OF FORMIC ACID.

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The detection and determination of small percentages of ethyl and methyl alcohol and of formic acid are often of considerable importance in food chemistry. Certain methods which appear to be of special value for this purpose are presented in this paper.

ETHYL ALCOHOL.

Ethyl alcohol, formed as a result of the action of the yeasts on sugars, is commonly found as a product of spontaneous fermentative changes in food products. Its presence in sufficient quantities in a fruit, a vegetable, or other food product, indicates decomposition at some time in the history of that substance. The mere detection of this alcohol, however, does not give much information as to the soundness of a food product, since traces of ethyl alcohol alone might not indicate a sufficient degree of chemical change in the fruit or vegetable to render it decomposed in the ordinary sense of the word. Further, mere traces of alcohol might have their origin in esters which give the aroma and taste to the fruit, and not be the result of a fermentation of the sugars present. It also appears to be a debatable proposition whether alcohol is not formed in saccharine solutions by many types of plants when kept under anaerobic conditions.[1]

It is evident, therefore, that not only the detection, but also the quantitative determination of alcohol is necessary to prove decomposition of the type represented by alcoholic fermentation. The amount of alcohol which may be present in such a decomposed food

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[1] Stoklasa, Ber. d. chem. Ges., 1903, **36**: 622· Chem. Centrbl., 1904, **1**: 961. Takahashi, Chem. Centrbl., 1902. **2**: 1330. Conheim, Zts. physiol. Chem., 1903, **39**: 348. Batteli, Compt. rend., 1902, **137**: 1029. Margnan, Compt. rend., 1905, **140**: 1124.

will probably vary from 0.1 to 1 per cent. An amount less than 0.1 per cent would, in the writer's opinion, call for further investigation to determine its significance; an amount greater than 1 per cent would probably represent a degree of decomposition such as to render the product of such an appearance as to be unsalable. The method used to estimate amounts of alcohol within these limits must be of such a nature as to leave no possible doubt that the substance determined is ethyl alcohol. Most of the present methods for detecting or determining small quantities of ethyl alcohol are weak at this point, being based on the quantity of potassium bichromate or other oxidizing agents which the alcohol will reduce. Such are the methods proposed by Bourcart,[1] Nicloux and Baudreur,[2] Benedict and Norris,[3] and others. It is evident from a careful study of these methods that slight changes in temperature (r concentration materially affect the accuracy of the results; indeed, Pozzi-Escot [4] claims that even when uniform conditions are rigidly maintained it is impossible to obtain accurate results for alcohol determinations by methods involving oxidation with potassium bichromate and sulphuric acid.

As very many substances reduce potassium bichromate, it is evident that in a determination based on that process there is no surety whatever that the substance so determined is ethyl alcohol. The method of Verley and Bölsing,[5] in which the acetic ester is prepared by the action on alcohols of acetic anhydrid in the presence of pyridin, is not accurate enough to be applied to very small quantities of alcohol, and it is evident that nearly any alcohol will give the same reaction.

As none of the proposed methods for determining alcohol when present in small amounts answers the requirements for accuracy and reliability of the work under the food and drugs act, studies to improve them were begun. The possibility of concentration of the alcohol from solutions containing only a small percentage of this substance was first considered. Nicloux and Baudreur [6] found that in distilling 600 cc solutions containing 0.2, 0.1, and 0.033 per cent of alcohol, respectively, from a liter distilling flask, there was obtained in the first 30 cc of distillate 50, 52, and 55 per cent of the total alcohol present. In repeating this work, using a Glinsky distilling head, the writer found that by these distillations it is only possible to obtain from 50 to 60 per cent of the total alcohol, when attempting to concentrate 0.1 per cent solutions into such a volume as to obtain solutions containing from 2 to 4 per cent of alcohol.

It was also found that if a considerable quantity of calcium or sodium chlorid was dissolved in the dilute alcohol solution before

[1] Zts. anal. Chem., 1890, **29**: 609.

[2] Bull. soc. chim., 1891, (3) **17**: 424.

[3] J. Amer. Chem. Soc., 1898, **20**: 293

[4] Ann. chim. anal., appl., 1907: 112.

[5] Ber. d. chem. Ges., 1901, **34**: 3354.

[6] Loc. cit.

distilling it, using an ordinary distilling flask, from 94 to 100 per cent of the total alcohol could be easily concentrated in a relatively small volume of distillate, as is shown from the following experiments selected from a number made to test this point:

Experiment 1.—To 500 cc of water containing 1 gram of absolute alcohol (0.2 per cent by weight) there were added 200 grams of calcium chlorid and this solution was distilled from a 1 liter distilling flask, 150 cc of distillate being collected.   To this distillate there were added 50 grams of calcium chlorid and a 50 cc solution was distilled from it. This distillate gave at 17.5° C., with the Zeiss immersion refracto-meter, a reading of 18.2, equivalent to 2 per cent of alcohol, a 100 per cent recovery.

Experiment 2.—Five hundred cubic centimeters of 0.19 per cent alcohol were treated as in experiment 1, save that of the first distillate but 100 cc were collected.   The refractometer reading at 17.5° C. was 17.9, equivalent to 1.84 per cent alcohol, a 96.8 per cent recovery.

Experiment 3.—This experiment was conducted exactly as in the case of No. 2, except that instead of using calcium chlorid, the solu-tions were about three-fourths saturated with common salt.   The refractometer reading was 17.9 at 17.5° C., equivalent to 1.84 per cent alcohol, a 96.8 per cent recovery.

Experiment 4.—From the distillates of experiments 2 and 3 (100 cc of 1.84 per cent alcohol) after three-quarters saturation with salt, there were distilled 50 cc.   The refractometer reading at 17.5° C. was 21.0, equivalent to 3.71 per cent alcohol (theory 3.68 per cent), a 100.8 per cent recovery.

Experiment 5.—Five hundred cubic centimeters of 1.6 per cent alcohol were treated as in experiment 3.   The refractometer reading at 17.5° C. was 42.7, equivalent to 15.02 per cent alcohol, a 94 per cent recovery.

Experiment 6.—One thousand cubic centimeters of 0.1 per cent alcohol were three-quarters saturated with salt, 150 cc were dis-tilled off, again three-fourths saturated with salt, and from this solu-tion 25 cc were distilled which at 17.5° C. had a refractometer reading of 21.3, equivalent to 3.84 per cent alcohol, a 96 per cent recovery.

These experiments prove that very small percentages of alcohol may easily be concentrated from salt solutions to a strength that can be used for the accurate determination of alcohol by known methods. The method here proposed for detecting and determining small quan-tities of alcohol is as follows:

Using the methods outlined in the preceding experiments con-centrate the sample until a solution containing from 2 to 4 per cent of alcohol is obtained.   If acids are present in the original solution exactly neutralize before distilling, using a trace of solid phenol-phthalein as an indicator.   Treat the final distillate as follows: Deter-

mine its specific gravity and refractometer reading at a suitable temperature, obtaining from tables[1] the corresponding percentages of alcohol.   If the refractometer reading and the specific gravity both correspond to the same percentage of alcohol, it is almost a certainty that it is ethyl alcohol that is present, and that the substance under examination contains the percentage of ethyl alcohol equivalent to these constants.   To render this an absolute certainty, the ethyl ester of paranitrobenzoic acid is prepared from this distillate with paranitrobenzoyl chlorid by the Baumann-Schotten method.[2]   To the solution containing the alcohol is added a slight excess of acid chlorid and an equivalent amount of sodium hydroxid.   Shake until the odor of the acid chlorid disappears.   This ester is a beautifully crystalline solid which may be readily identified by its melting point of 57° C.[3]   This ester may be weighed, and while the yield obtained is not quite quantitative (70 to 90 per cent of the theory when working with small quantities of alcohol), its weight serves as another check on the percentage of alcohol present in the solution.   If para-nitrobenzoyl chlorid is not available the benzoic acid ethyl ester may be prepared as just described, using benzoyl chlorid.   The benzoic acid ethyl ester may be weighed (yield practically quantitative), and identified by its odor and its boiling point, 212° C., using for the latter purpose the convenient method for determining the boiling point of very small quantities of liquids recently proposed by Smith and Menzies.[4]

As to the qualitative detection of ethyl alcohol, two tests used together, the formation of iodoform and the formation of ethyl benzoate would render the presence of ethyl alcohol probable, for the only benzoic ester whose odor is similar to that of the ethyl ester is the methyl ester, and methyl alcohol does not give the iodoform reaction.[5]   A little careful consideration of the question, however, will make it evident that for an absolutely unassailable result, when ethyl alcohol is present in small amounts, some such method as that just outlined for the determination of this substance must be used. After the alcohol is concentrated as described, other confirmatory tests, such as the determination of the freezing point, the boiling point, the capillarity constant, the electrical conductivity, or the preparation of other esters, may be applied to the liquid.

It has been attempted to describe in a general way such tests as can be easily made and which prove absolutely that an aqueous liquid contains a definite quantity of ethyl alcohol.   It is realized,

[1] Tables of Ackermann and Steinmann, Zts. gesam. Brauwesen, 1905, 28: 259; Doroszewski and Swor-zanczyk, J. Russ. Phys. Chem. Soc., 1907, 40: 101-125.

[2] Ber. d. chem. Ges., 1884, 17 : 2445 ; 19: 3219.

[3] Ber. d. chem. Ges., 1905, 38 : 620.

[4] J. Amer. Chem. Soc., 1910, 32 : 897.

[5] The limit of these reactions is about 0.1 per cent of ethyl alcohol.

however, that there are many mixtures from which the alcohol can not be separated by the general method here given, and such special cases must be treated individually. After the alcohol is separated by some method applicable to the mixture in hand the general methods herein described may be applied. They have been used in this laboratory by H. C. Gore in following the decomposition changes in bananas, with quite satisfactory results.

METHYL ALCOHOL.

A convenient and reliable method of detecting methyl alcohol is that of Mulliken and Scudder,[1] in which the alcohol is oxidized to formaldehyde by means of a spiral of oxidized copper wire. A careful study of this method showed that to obtain a positive test it is necessary to have a solution containing over 0.1 per cent of methyl alcohol. It seemed that greater delicacy would be obtained in such a test by conducting the oxidation with certain substances in solution instead of using the solid copper oxid in wire form as proposed by Mulliken.[2] A. Vorisek[3] proposed such a method, but as he did not determine its delicacy and the apparatus used by him seemed cumbersome, the subject was further studied. The method proposed by the writer is as follows:

To 100 cc of aqueous methyl alcohol in a 200 cc distilling flask add from 5 to 8 grams of chromic acid; collect 10 cc of distillate and test for formaldehyde by the Leach or Hehner methods.[4] If other oxidizable substances, such as acids, sugars, starches, proteids, etc., are present the solution under examination is exactly neutralized with sodium hydroxid and one-third of it is distilled, the distillate being oxidized and redistilled as has just been described. The use of the relatively large quantity of chromic acid indicated is found to increase the delicacy of the reaction. By the proposed method it is very easy to detect one part of methyl alcohol in 100,000 parts of water, and by making one preliminary distillation to concentrate the methyl alcohol, collecting the first 100 cc from a liter and then proceeding as before described, a very strong test was obtained from a solution containing only one part of methyl alcohol per million.

When testing for methyl alcohol in the presence of strong ethyl alcohol it is advisable to dilute the alcohol with water to obtain approximately a 20 per cent solution, as otherwise the action of the chromic acid on the ethyl alcohol may become explosively violent. It is easy by this method to detect 0.1 per cent of methyl alcohol in 80 per cent alcohol, and by a little fractionation it was found possible

[1] J. Amer. Chem. Soc., 1899, **21**: 266.

[2] Loc. cit.

[3] J. Soc. Chem. Ind., 1909, **28**: 823.

[4] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised, p. 185; Analyst, 1895, **20**: 155.

to detect 0.01 per cent in 80 per cent ethyl alcohol.   Potassium permanganate may be substituted for chromic acid in this oxidization, but the sensitiveness of the reaction for aqueous solutions when using this reagent is then only about 1 part in 4,000.   For the determination of methyl alcohol the same principles and method given under ethyl alcohol apply.

FORMIC ACID.

The detection and determination of formic acid in food products is becoming of increasing importance, not only because of the proposed use of this acid as a preservative but also because it has been found to be one of the products of the fermentative action of bacteria and yeasts on carbohydrates.   Fenton [1] has shown that carbonic and formic acids in aqueous solution may be reduced to formaldehyde by the action of magnesium.   The reduction of formic acid has been studied in this laboratory to determine its sensitiveness, and certain other metals have been found to give the same reduction.   The formation of formaldehyde from carbon dioxid by these metals is of considerable theoretical importance; but, in the practical method of testing for formic acid, as proposed here, there is no danger of confusing this acid with carbonic acid, as to obtain detectable quantities of formaldehyde from carbon dioxid it is necessary to cause this gas to act on the metal for several hours.

If a suspected solution is acidified with phosphoric acid and then distilled the amount of carbon dioxid found in the distillate is not sufficient in amount to give detectable quantities of formaldehyde when reduced by metals for any reasonable time.   In the following table the action of various metals on 1 per cent and 0.1 per cent formic acid solutions is given, the reduction to formaldehyde being indicated as positive and negative:

The reduction of formic acid by the action of various metals.

Metals.	1 per cent formic acid.	0.1 per cent formic acid.
Magnesium filings...	Positive............	Positive.
Calcium metal..	.....do..............	Do.
Coppered zinc filings...	.....do..............	Do.
Coppered zinc dust...	.....do..............	Do.
Zinc amalgam..	Negative...........	
Coppered aluminum..	.....do..............	Negative.
1 per cent sodium amalgam...................................	.....do..............	
1 per cent sodium amalgam with magnesium chlorid solution ...	.....do..............	
Aluminum...	.....do..............	
Aluminum amalgam..	Faintly positive...	Do.
Zinc dust..	....	Do.
Zinc filings..	....	Do.

[1] J. Chem. Soc. (Lond.), 1907, **91**: 687.

As magnesium filings seem to give the best results the reaction is carried out as follows:

Strongly acidify the solution with phosphoric acid and distill about one-third of it. To the distillate add dilute sulphuric acid and magnesium filings in sufficient quantities to cause a vigorous but not a violent evolution of hydrogen. (In case quite a large quantity of acid is present in the distillate it is not necessary to add any sulphuric acid.) If the amount of formic acid is small (about 0.1 per cent) continue the action for one hour. If larger quantities are present, the test for formaldehyde will usually be obtained in from two to five minutes. Of course the absence of formaldehyde in the original solution must be determined.

The common methods for the determination of formic acid are based on the following general reactions: (1) The reduction of a mercuric salt to a mercurous salt, the insoluble mercurous salt being weighed; (2) the reduction of potassium permanganate in alkaline solution; (3) the action of concentrated sulphuric acid, the evolved carbon monoxid being measured. With regard to methods 1 and 2, aldehydes, certain alcohols, and many other volatile substances which may be present in fruits and vegetables effect the same reduction as formic acid. With regard to method 3, a very large number of organic acids evolve carbon monoxid when treated with concentrated sulphuric acid.

While platinic salts are readily reduced to metal by many aldehydes and other reducing agents in alkaline solution, it was found that of a great number of substances tried only formic acid accomplished this reduction in neutral or weak acid solutions. The following method is therefore proposed: Distill the solution containing the formic acid with a small quantity of phosphoric acid until the distillate is no longer acid. If the volume of this distillate is too large to be conveniently handled, neutralize it with sodium hydroxid and evaporate to a convenient volume. Add an excess of platinic chlorid and sufficient acetic acid to make the solution strongly acid (usually about 1 or 2 cc of glacial acetic acid for less than 1 gram of formic acid), and boil the solution for one hour, using a reflux condenser. Collect the reduced platinum in the usual manner and weigh. The weight of the platinum multiplied by 0.472 equals the formic acid present.

The following are examples of results obtained by this method:

(1) A 100 cc solution containing 0.0782 gram of formic acid; platinum reduced, 0.160 gram = formic acid 0.0755 gram; 97 per cent of theoretical amount.

(2) A 100 cc solution containing 0.243 gram of formic acid; platinum reduced, 0.504 gram = 0.238 gram of formic acid, 98 per cent recovery.

(3) A 100 cc solution containing 0.243 gram of formic acid; platinum reduced, 0.517 gram = 0.244 gram of formic acid, 100.4 per cent recovery.

(4) A 100 cc solution containing 0.1215 gram of formic acid; platinum reduced, 0.255 gram = 0.120 gram of formic acid, 98 per cent recovery.

The electric conductivity of formic acid is so much greater than that of other similar organic acids that it seems possible to base a method for its determination on that property. A further study of this problem is in progress.

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